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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b> <b>A61L 27/00, A61K 31/54</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 90/06138</b> <b>(43) International Publication Date:</b> 14 June 1990 (14.06.90)
<b>(21) International Application Number:</b> PCT/GB89/01423 <b>(22) International Filing Date:</b> 28 November 1989 (28.11.89)  <b>(30) Priority data:</b> 8827986.4 30 November 1988 (30.11.88) GB  <b>(71) Applicant (for all designated States except CH US):</b> HOLMES, Michael, John [GB/GB]; Frank B. Dehn & Co., Imperial House, 15-19 Kingsway, London WC2B 6UZ (GB).  <b>(71) Applicant (for all designated States except US):</b> ED GEIST- LICH SÖHNE A.G. FÜR CHEMISCHE INDUSTRIE [CH/CH]; Wolhusen, CH-6110 Lucerne (CH).  <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> PFIRRMANN, Rolf, Wil- helm [CH/CH]; Schadrudstrasse 27, CH-6006 Lucerne (CH).		<b>(74) Common Representatives:</b> HOLMES, Michael, John et al.; Frank B. Dehn & Co., Imperial House, 15-19 Kingsway, London WC2B 6UZ (GB).  <b>(81) Designated States:</b> AT (European patent), BE (European patent), CH, DE, ES (European patent), FR (European patent), GB, IT (European patent), JP, LU (European patent), NL, SE (European patent), US.  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> IMPLANT FOR USE IN BONE SURGERY		
<b>(57) Abstract</b>  A lyophilised collagen sponge for use as an implant in osteitis and other bone cavities, said sponge having dispersed there- in antibacterially effective quantities of taurolidine and/or taurultam.		

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IMPLANT FOR USE IN BONE SURGERY

5        This invention relates to a novel collagen- based sponge material for use as an implant in bone surgery.

      The treatment of osteomyelitis and osteitis is notoriously difficult. It is necessary to remove all infected bone material and then to induce remodelling, that is re-growth of healthy bone tissue, within the cavity as formed. Unfortunately, re-infection is common and it is necessary that a powerful antibacterial substance is present during the remodelling phase. Remodelling in facio-maxillary surgery and tooth  
10        extraction socket filling imposes similar requirements.

      In our European Patent No. 48558 we have described resorbable aqueous gels comprising cross-linked gelatin or collagen materials containing antibiotics such as gentamycin or more preferably, taurolidine, for  
20        implantation into osteitis cavities. While such gels have proved successful for most purposes, there is a need for alternative implant materials, particularly for implantation into cavities in small bones such as those of the fingers and toes, as well as tooth  
25        extraction cavities and other relatively small cavities.

      We have now found that a lyophilised sponge of collagen fibres containing the antibacterial substances taurolidine and/or taurultam provide an extremely effective implant material for such uses. Although  
30        collagen sponges have been proposed which contain cross-linked or otherwise pre-treated collagen fibres together with an antibiotic such as gentamycin, it has not previously been proposed to impregnate such sponges with taurolidine and taurultam, which have particular  
35        advantages in treating bone cavities and bone injuries generally.

      According to the present invention we provide a

lyophilised collagen sponge for use as an implant in osteitis and other bone cavities, said sponge having dispersed therein antibacterially effective quantities of taurolidine and/or taurultam.

5       Taurolidine and taurultam are methylol transfer agents which are able to combat not only gram negative and gram positive bacteria but also the exotoxins and endotoxins they produce. They are thus particularly well suited for the treatment of bone cavities liable to  
10 re-infection. The soluble collagen sponge according to the invention releases the active substances firstly by diffusion and then by dissolution or resorption of the collagen. The sponge conveniently contains from 1-30 mg/cm<sup>2</sup> of taurolidine and/or from 1-60 mg/cm<sup>2</sup> taurultam.

15       Collagen fibres are the most common type of fibres in the connective tissue and the commonest protein in the human body, corresponding to 30% of the total protein. The hyaline cartilage material of the bone consists of 40-45% of collagen fibres. Human bone  
20 contains about 40g collagen nitrogen per kg. Collagen fibres consist of collagen fibrils having diameters of 0.2 to 0.5 microns. Their peptide structure contains a high level of proline (12%) and hydroxyproline (10%) residues. Each fibril consists of overlapping molecules  
25 of tropocollagen each of which includes a superhelix of 3 polypeptide alpha- chains, which are interwound and stabilised by hydrogen bonding and have terminal non-helical telopeptide sequences.

30       Four types of collagen are recognised, in which the tropocollagen is built up from three different polypeptide alpha-chains with an average molecular weight of 100,000. The commonest is Type I, occurring for example in skin, muscle, bone, tendons and fascia, which consists of two identical alpha-1-chains and one  
35 alpha-2- chain with a different amino acid sequence. Types II, III, and IV consist of three alpha-1-chains which differ in their primary structure in different

parts of the body. Type II is the most common collagen type in the hyaline cartilage. Type III occurs inter alia, in the blood vessels and in foetal membranes. Type IV occurs in the basal laminae.

5        There are significant differences between collagen fibres at different conditions of maturity. Where the connective tissue is in an active phase of fibrillogenesis, for example during growth or wound healing, collagen fractions can be isolated with different  
10 properties. The first fraction is extractable by neutral solutions (neutral-soluble collagen); this consists of recently synthesised tropocollagen molecules which are not aggregated or are only beginning to aggregate. The second fraction is extractable by a  
15 sodium citrate solution at pH 3.0, and is thus termed the acid-soluble collagen fraction. The third fraction found in older tissues is the insoluble fraction and can only be extracted by very vigorous methods. One basis for the difference between these fractions lies in the  
20 degree of cross-linking by oxidation to produce peroxide bridges. Collagen can also be cross-linked chemically via free amino groups, using aldehydes such as formaldehyde or glutaraldehyde or isocyanates such as hexamethylene diisocyanate. By such cross-linking,  
25 animal collagen fractions lose their antigenicity almost completely. Cross-linking of collagen fibrils in this way is for example, made use of in heart replacement surgery, where animal, e.g. porcine, valves are conditioned with glutaraldehyde for use as human  
30 pulmonary or mitral valve replacements.

In general, it is preferred that the collagen is water insoluble but is rapidly resorbed eg within up to 12 hours, for example within 6 to 12 hours. This is compatible with the relatively short half-life of  
35 taurolidine and taurultam. Aged or acid soluble collagen may thus be used or, more preferably, neutral soluble collagen fibres may be artificially aged by

oxidation, eg using a peroxide such as hydrogen peroxide, to form oxygen bridges. Collagen of type I, especially from skin and tendons, advantageously from the flank skin of young calves, is preferred.

- 5        However, it may be beneficial to lightly cross-limits neutral soluble collagen, eg by treatment with a cross-linking agent, for example an aldehyde such as formaldehyde or glutaraldehyde or a isocyanate such as hexamethylene diisocyanate. Such a cross-linked form  
10 of collagen will be resorbed more slowly and thus may release the taurolidine or taurultam over a longer period. It is particularly preferred, however, that the level of any cross-linking is much that the collagen is resorbed in 12 hours or less after implantation.

- 15        Where the collagen is cross linked it may be beneficial to include an emulsifying agent, during the foaming and lyophilising step eg lecithin and/or Cremophor EL (available from BASF), both of which are parenterally acceptable.

- 20        Suitable collagen sponge may be obtained commercially, for example from Pentapharm AG of Basel, Switzerland, from Dr Otto Suwelak GmbH of Billerbeck, West Germany or from Ed Geistlich Söhne A.G. of Wolhusen, Switzerland. Alternatively, such material may  
25 be obtained from the appropriate tissues by conventional methods.

- Thus, for example, bovine skin, advantageously from young calves, and preferably from the flank region, may be chemically dehaired and mechanically split to  
30 separate off the epidermis and the underskin with associated fat. It is important to avoid or minimise contamination with fat. The layer so obtained may be treated with mild alkali, such as calcium hydroxide, eg for about 4 weeks. The resulting mat rial may then be  
35 acidified, eg with 3% hydrochloric acid, washed with running water and comminuted. A proteolytic enzyme may be used to assist separation of collagen from other

proteins and a lipase may be used to remove residual fat. However, it is important to avoid antigenic reactions which may result from the use of such enzymes.

The neutral-soluble collagen so produced may then  
5 be treated with an oxidising agent such as hydrogen peroxide to form oxygen bridges similar to those formed in the natural ageing of collagen.

The comminuted product may then be homogenised with about 7 parts by weight of water, the pH adjusted to  
10 about 5.3 and the product further homogenised to produce a foam.

The foamed homogenised material is then filled into cooling cells, e.g. to a depth of about 1.5 cm, rapidly cooled to -20°C and lyophilised.

15 The incorporation of the taurolidine or taurultam may be effected either by foaming a collagen solution containing e.g. 2% taurolidine or taurultam, prior to lyophilisation or by redissolving lyophilised collagen in a solution of taurolidine or taurultam and  
20 re-lyophilising.

The lyophilised collagen sponge material will normally be sealed in plastic containers and sterilised by radiation e.g. gamma radiation.

25 Sheets of the collagen sponge according to the invention may be conveniently about 0.5 cm in thickness. Such sheets can be readily cut by the surgeon into small shaped pieces for use as implants. They will normally be laid into the bone cavity without compression. If  
30 necessary, spongeosa may also be introduced into the cavity at the same time.

The invention is illustrated by the following non-limiting Examples. Collagen GN is available from Ed. Geistlich Söhne A.G.

Example 1

Collagen GN (a fleecy material containing some collagen fibres,  $21 \times 29.8 \text{ cm} = 625.8 \text{ cm}^2$ ) is soaked with 260g of a  
5 4.8% (w/w) taurolidine solution and then immediately frozen. Freeze-drying gives a compact taurolidine-collagen sponge with 20 mg taurolidine/cm<sup>2</sup>.

Example 2

10

Collagen GN ( $21 \times 29.8 \text{ cm} = 625.8 \text{ cm}^2$ ) is soaked with 260g of a 4.8% (w/w) taurolidine solution, immediately frozen and then freeze-dried. The dried material is soaked a  
15 second time with 130g of a 4.8% (w/w) taurolidine solution and freeze-dried to give a compact taurolidine-collagen sponge with 30 mg taurolidine/cm<sup>2</sup>.

Example 3

20 Collagen GN ( $21 \times 29.8 \text{ cm} = 625.8 \text{ cm}^2$ ) is soaked with 250g of a 15% taurultam solution and immediately frozen. Freeze-drying gives a compact taurultam-collagen sponge with 60 mg taurultam/cm<sup>2</sup>.

25 Example 4

Collagen GN ( $21 \times 29.8 \text{ cm} = 625.8 \text{ cm}^2$ ) is soaked with 287.5g of a 13.05% taurultam solution and immediately  
frozen. Freeze-drying gives a soft taurultam-collagen  
30 sponge with 60 mg taurultam/cm<sup>2</sup>.

Example 5

Collagen GN ( $21 \times 29.8 \text{ cm} = 625.8 \text{ cm}^2$ ) is soaked with  
35 537.5g of a 7% taurultam solution and immediately frozen. Freeze-drying gives a soft, downy taurultam-collagen sponge with 60 mg taurultam/cm<sup>2</sup>.

Claims:

1. A lyophilised collagen sponge for use as an implant  
in osteitis and other bone cavities, said sponge having  
5 dispersed therein antibacterially effective quantities  
of taurolidine and/or taurultam.
2. A collagen sponge as claimed in claim 1 wherein the  
collagen is selected from acid soluble collagen and  
10 artificially aged neutral soluble collagen, whereby the  
resulting implant is capable of resorption in the human  
body within up to 12 hours.
3. A collagen sponge as claimed in claim 1 or claim 2  
15 wherein the collagen comprises type I collagen.
4. A process for the preparation of a collagen sponge  
as defined in claim 1 in which a solution or suspension  
of collagen fibres in an aqueous solution of taurolidine  
20 or taurultam is lyophilised.
5. Use of a collagen sponge as defined in claim 1 in  
surgery.

# INTERNATIONAL SEARCH REPORT

International Application No **PCT/GB 89/01423**

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>4</sup> According to International Patent Classification (IPC) or to both National Classification and IPC <b>IPC5: A 61 L 27/00, A 61 K 31/54</b>														
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched <sup>7</sup></div> <div style="display: flex; justify-content: space-between;"> <span>Classification System</span> <span>Classification Symbols</span> </div> <div style="margin-top: 10px;"> <b>IPC5</b>      <b>A 61 L; A 61 K</b> </div> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin-top: 10px;">Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched <sup>8</sup></div>														
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b> <table border="1" style="width: 100%; border-collapse: collapse; margin-top: 5px;"> <thead> <tr> <th style="width: 10%;">Category <sup>10</sup></th> <th style="width: 60%;">Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup></th> <th style="width: 30%;">Relevant to Claim No. <sup>13</sup></th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top;">A</td> <td style="vertical-align: top;">EP, A2, 0048558 (ED. GEISTLICH SÖHNE A.G. FÜR CHEMISCHE INDUSTRIE) 31 March 1982, see the whole document  --</td> <td style="text-align: center; vertical-align: top;">1-5</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">A</td> <td style="vertical-align: top;">US, A, 4789663 (D.G. WALLACE ET AL.) 6 December 1988, see the abstract, column 4, lines 1-17, lines 31-55, claims 1-5  --</td> <td style="text-align: center; vertical-align: top;">1-3,5</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">A</td> <td style="vertical-align: top;">WO, A1, 86/07265 (ED. GEISTLICH SOHNE A.G. FÜR CHEMISCHE INDUSTRIE) 18 December 1986, see the whole document  --</td> <td style="text-align: center; vertical-align: top;">1-5</td> </tr> </tbody> </table>			Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>	A	EP, A2, 0048558 (ED. GEISTLICH SÖHNE A.G. FÜR CHEMISCHE INDUSTRIE) 31 March 1982, see the whole document  --	1-5	A	US, A, 4789663 (D.G. WALLACE ET AL.) 6 December 1988, see the abstract, column 4, lines 1-17, lines 31-55, claims 1-5  --	1-3,5	A	WO, A1, 86/07265 (ED. GEISTLICH SOHNE A.G. FÜR CHEMISCHE INDUSTRIE) 18 December 1986, see the whole document  --	1-5
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>14</sup> Special categories of cited documents: <sup>15</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>														
<b>IV. CERTIFICATION</b> <table style="width: 100%; margin-top: 5px;"> <tr> <td style="width: 50%; vertical-align: top;">           Date of the Actual Completion of the International Search  <b>26th February 1990</b> </td> <td style="width: 50%; vertical-align: top;">           Date of Mailing of this International Search Report  <b>19. 03. 90</b> </td> </tr> <tr> <td style="vertical-align: top;">           International Searching Authority  <b>EUROPEAN PATENT OFFICE</b> </td> <td style="vertical-align: top;">           Signature of the International Searching Authority  <div style="text-align: right; margin-top: 10px;"><b>T.K. WILLIS</b></div> </td> </tr> </table>			Date of the Actual Completion of the International Search <b>26th February 1990</b>	Date of Mailing of this International Search Report <b>19. 03. 90</b>	International Searching Authority <b>EUROPEAN PATENT OFFICE</b>	Signature of the International Searching Authority <div style="text-align: right; margin-top: 10px;"><b>T.K. WILLIS</b></div>								
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	<p>EP, A2, 0139534 (ED. GEISTLICH SÖHNE A.G. FÜR CHEMISCHE INDUSTRIE) 2 May 1985, see the abstract, page 4, lines 30-34, claims</p> <p style="text-align: center;">-----</p>	1

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

**PCT/GB 89/01423**

**SA 32683**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0048558	31/03/82	JP-A- 57077616	15/05/82
		AU-D- 74861/81	11/03/82
		CA-A- 1190855	23/07/85
		US-A- 4587268	06/05/86
		AU-A- 554672	28/08/86
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US-A- 4789663	06/12/88	EP-A- 0171176	12/02/86
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		AU-D- 17515/88	27/10/88
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		JP-A- 60105617	11/06/85
		DE-A- 3438470	30/05/85
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		CH-A- 660969	30/06/87
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